

FIELD ATTRACTION OF MEDITERRANEAN FRUIT FLY, *Ceratitis capitata* (WIEDEMANN) TO SYNTHETIC STEREOSELECTIVE ENANTIOMERS OF THE CERALURE B1 ISOMER

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Abstract—Stereoselectively synthesized enantiomers of ethyl *cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate (ceralure B1), a potent lure for male Mediterranean fruit flies, were tested in the laboratory and the field against laboratory reared sterile flies. The (–)-ceralure B1 enantiomer was significantly more attractive than the (+)-ceralure B1 antipode. Dose–response studies of the above compounds demonstrated that (–)-ceralure B1 and to a lesser extent, racemic ceralure B1, captured consistently more male flies than trimedlure, the current male attractant used in detection programs. Longevity tests demonstrated that, over a three-week period, both (–)-ceralure B1 and racemic ceralure B1 caught significantly more flies than trimedlure. The synthesis of specific enantiomers of ceralure B1 shows great promise as a male attractant that could replace trimedlure for detection and delimitation in action programs aimed at this exotic pest.

Key Words—Mediterranean fruit fly, attractants, detection, male annihilation.

INTRODUCTION

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Wiedemann) is a serious pest of fruits and vegetables throughout many parts of the world. Except for the state of Hawaii, medfly is not established in the United States. If established, this pest would have a serious impact on US agriculture and the environment through increased costs for control and additional pesticide sprays. In addi-

tion, the regulatory quarantines by importing countries could severely restrict worldwide trade of US agricultural products (CDFA, 1994; Siebert and Cooper, 1995).

The establishment of this pest within the contiguous United States has been prevented through a vigorous detection, quarantine inspection, and eradication program. Monitoring and detection for the medfly using synthetic attractants is a key step in signaling the need for eradication. Each year states such as California, Florida, and Texas employ more than 100,000 traps in and around agricultural and urban areas to survey and detect possible introductions of medfly. For almost 40 years, trimedlure (TML), developed from an intensive US Department of Agriculture screening and testing program has been widely used as the "standard" synthetic male medfly attractant (Beroza et al., 1961). For commercial use, 2 g of trimedlure is formulated in a polymeric plug-type dispenser that provides controlled release of the attractant for up to eight weeks when deployed in Jackson traps (Leonhardt et al., 1987, 1989; Gilbert and Bingham, 1999).

Commercial trimedlure (TML) is an isomeric mixture of *tert*-butyl esters of 4- and 5-chloro-2-methylcyclohexane-1-carboxylate. Hence, trimedlure is a mixture of 16 possible regio- and stereoisomers. Of these, a single isomer (1*S*,2*S*,4*R*)-*tert*-butyl 4-chloro-2-methylcyclohexane-1-carboxylate (trimedlure-C enantiomer, henceforth referred to as (+)-trimedlure-C based on its optical rotation) was found to be the most attractive (McGovern et al., 1987; Doolittle et al., 1991; Heath et al., 1990). Furthermore, the optical antipode, (–)-trimedlure-C ((1*R*,2*R*,4*S*)-*tert*-butyl 4-chloro-2-methylcyclohexane-1-carboxylate) was shown to have very little biological activity.

McGovern co-workers investigated various halogen and ester analogs of trimedlure and found that ethyl 4- (and 5-) iodo-*trans*-2-methylcyclohexane-1-carboxylate (ceralure) was more potent and persistent than trimedlure (McGovern and Cunningham, 1988; DeMilo et al., 1994; Warthen et al., 1994). Like trimedlure, commercial ceralure is also a mixture of 16 regio- and stereoisomers. A field study designed to measure the relative attractiveness of the racemates, which were tediously separated by HPLC, demonstrated that racemic ethyl *cis*-5-iodo-*trans*-2-methylcyclohexane-1-carboxylate (ceralure B1) is most active (Warthen et al., 1994). Recently, a highly effective stereoselective synthesis that yields the two enantiomers of ceralure B1 on a multigram scale was developed (Raw and Jang, 2000).

For the current study, laboratory and field tests with released sterile flies on the island of Hawaii were carried out to compare the attractancy, dose–response, and initial persistence of the pure enantiomers as well as the racemic mixture. The purpose of this study was to evaluate the relative attractancy of the two nearly synthesized ceralure B1 enantiomers and to compare these to responses of commercially available trimedlure and ceralure.

METHODS AND MATERIALS

Test Compounds. Liquid trimedlure (UOP Chemicals, East Rutherford, New Jersey) (98% pure; density 1.02 g/ml) and ceralure (Agrisense/Biosys, Palo Alto, California) (98% pure; density 1.43 g/ml) were purchased from commercial sources. Ceralure was stored over a copper coil to prevent discoloration. The enantiomers of ceralure B1, ethyl (1*R*,2*R*,3*R*)-5-iodo-2-methylcyclohexane-1-carboxylate and ethyl (1*S*,2*S*,3*S*)-5-iodo-2-methylcyclohexane-1-carboxylate, henceforth referred to as (–)-ceralure B1 and (+)-ceralure B1, respectively, based on their optical rotations, were synthesized by using a unique nine-step process (Raw and Jang, 2000). This yielded each enantiomer on a multigram scale with high purity (97% ee) and an overall yield of 15%. Racemic ceralure B1 was synthesized via the same synthetic scheme utilized to synthesize the pure enantiomers of ceralure B1, with the exception of using racemic sigluric acid as starting material.

Insects. Sterilized medfly pupae were obtained from the USDA-ARS Pacific Basin Agricultural Research Center (PBARC) rearing facility in Honolulu, Hawaii. The flies were reared on an artificial diet (Tanaka et al., 1969) under standard mass rearing conditions and sterilized as pupae at a dose of 150 Gy two days before emergence. Flies were shipped by air to the PBARC laboratories in Hilo, HI, where 60–150 ml of pupae (both sexes) were placed either in small aluminum cubical screen cages (0.3 × 0.3 × 0.3 m) or in larger holding cages made from 57-liter (15-gallon) Rubbermaid “action packers” (Rubbermaid Inc., Khartoum, Sudan) modified by screened panels on the bottoms and sides of the units. The emerged flies were given water, sugar and hydrolyzed yeast protein. Flies were 2–9 days old when tested.

Rotating Olfactometer Test. Initial tests were conducted in a four-arm outdoor rotating olfactometer (3 × 3 × 2.5 m) as described in Jang et al. (1997). Neat compounds were diluted in reagent-grade acetone to give the following doses: 10, 1, 0.1, and 0.01 μg (in 100 μl acetone). The solutions were pipetted onto a 1.2-cm cotton wick inside of a Jackson trap containing a cardboard insert coated with stickem glue. One cubical aluminum cage of medflies (approximately 3600 flies of mixed sex) was released into the olfactometer. Temperature ranged from 20 to 24°C and relative humidity was 65–88%. Traps were allowed to slowly rotate within the olfactometer cage for 4 hr. At the end of the 4-hr test period, traps were removed and trapped flies were counted and recorded.

Open Field Test. All field tests were conducted by using Jackson traps with a 1.2-cm-long × 0.7-cm-diameter cotton wick that contained the test chemical (Harris et al., 1971). All tests were conducted with a randomized complete block design having five traps of each treatment in the block. The tests were replicated at least five times.

Dose–Response Tests. Traps were baited with (+)-ceralure B1, (–)-ceralure

B1, racemic ceralure B1, commercial trimedlure, commercial ceralure, or a blank (acetone) control. For each compound, the following doses were tested: 10, 1, 0.1, and 0.01 mg. Traps were placed in a macadamia nut orchard on the Island of Hawaii near the town of Hilo. Traps were separated by every other tree in every row (approximately 10 m between traps). Flies (approximately 6000/row) were then released by slowly walking between rows while allowing the flies to escape from the opened action packer. After 48 hr, traps were collected and brought back to the lab for analysis. Both males and females medflies were counted on the sticky inserts.

Longevity Tests. Preliminary tests to determine the effectiveness of the different chemical compounds over time were also carried out using sterile flies released in a macadamia nut orchard. In these tests, all compounds were applied to the cotton wick at a 10 mg dose. Traps were serviced weekly for three weeks. Inserts were changed each week and new flies were released, but the same cotton wicks were left in the field to age throughout the 3-week test period.

Data Analysis. Although male and female medflies were counted on the sticky inserts, the trap captures were overwhelmingly male biased. Thus, only data from male captures are included in the analysis. Data were subjected to analysis of variance (Proc GLM) and Tukey's honestly significant difference test for separation of mean values. Significant differences in response were determined at the $P < 0.05$ level. Data were not transformed prior to analysis. Statistical analysis was performed on SAS ver 6.12 (SAS Institute, Cary, North Carolina) with a PC. Values presented in the tables are means \pm SEM.

RESULTS

In outdoor olfactometer cage tests, traps baited with (–)-ceralure B1 captured numerically more male medflies than did other traps at all concentrations. In comparison with commercial ceralure and the control, (–)-ceralure B1 produced significantly greater capture at the 10- and 1- μ g mg dosages. At the 1- μ g dosage, (–)-ceralure B1 was also different from racemic ceralure B1. There were no significant differences in trap captures among compounds at the 0.01- μ g dosage (Table 1).

The field results comparing the relative attractancy of optically active ceralure B1 enantiomers, racemic ceralure B1, commercial trimedlure, and commercial ceralure at various doses are shown in Table 2. Traps baited with (–)-ceralure B1 captured significantly more male medflies than those baited with its (+)-ceralure B1 antipode. In addition, (–)-ceralure B1 and racemic ceralure B1 produced significantly higher capture than commercial trimedlure or ceralure at all dosages. In the course of these studies, it was noted that, at both the 10-mg and 1.0-mg doses of (–)-ceralure B1, trap saturation (overcrowding) was a

TABLE 1. RESPONSE OF RELEASED MEDITERRANEAN FRUIT FLIES TO DIFFERENT DOSAGES OF TML AND CERALURE COMPOUNDS IN ROTATING OLFACTOMETER^a

Treatment	N	Male fly capture (mean ± SE)			
		10 µg ^b	1 µg	0.1 µg	0.01 µg
TML	4	211.5 ± 11.5 abc	176.2 ± 35.5 ab	91 ± 23.4 ab	4.5 ± 0.8 a
Com. Ceralure	4	87.5 ± 0.5 bc	42.5 ± 10 c	92 ± 25.3 ab	3.5 ± 1.5 a
(+)-Ceralure B1	4	241.5 ± 28.5 ab	234.2 ± 27.7 ab	131 ± 9.7 ab	8 ± 2.2 a
(±) Ceralure B1	4	188 ± .5 abc	131 ± 30.6 bc	121.5 ± 9.8 ab	7.2 ± .8 a
(-)-Ceralure B1	4	275 ± 16 a	296.7 ± 31.8 a	161.5 ± 38.5 a	11.5 ± 3.4 a
Control	4	50 ± 30 c	48 ± 15.2 c	38.5 ± 4.3 b	7.2 ± 1.6 a

^aData analyzed by Proc GLM; means followed by different letters in a column are significantly different (*P* < 0.05) by Tukey's test.

^bTwo replicates.

concern. Thus, the data at those concentrations may not be indicative of the relative attractiveness of (-)-ceralure B1 to the other attractants (e.g., trimedlure or commercial ceralure). At the 0.1-mg and 0.01-mg doses, trap saturation was not an issue. At these dosages, medfly capture by (-)-ceralure B1 outperformed, on average, commercial ceralure by 16-fold, and trimedlure by ninefold.

In longevity studies, all compounds were tested at the 10-mg dose. During the first week, both (-)-ceralure B1 and racemic ceralure B1 captured significantly more males than (+)-ceralure B1, commercial trimedlure, commercial ceralure, and control (Table 3). During the second week, captures at traps baited with (-)-ceralure B1 and racemic ceralure B1 were 20% and 30%, respectively, of the initial response before aging, whereas capture at traps baited with (+)-

TABLE 2. FIELD RESPONSE OF RELEASED MEDITERRANEAN FRUIT FLIES TO DIFFERENT DOSAGES OF TML AND CERALURE COMPOUNDS^a

Treatment	N	Male fly capture (mean ± SE)			
		10 mg	1 mg	0.1 mg	0.01 mg ^b
TML	15	133.3 ± 30.7 c	112.5 ± 20.9 bc	31.1 ± 12.4 c	14 ± 3.4 bc
Com. Ceralure	15	170.5 ± 33.1 c	65.1 ± 9.5 bc	11.3 ± 4.1 c	23.4 ± 6.3 bc
(+)-Ceralure B1	15	151.8 ± 25.3 c	173 ± 42.2 b	57.2 ± 8.3 c	38.3 ± 6.5 b
(±)-Ceralure B1	15	365.5 ± 33.7 b	376.8 ± 36.7 a	179.7 ± 33.5 b	111 ± 12.2 a
(-)-Ceralure B1	15	487.3 ± 33 a	473.1 ± 56.6 a	303.1 ± 34.5 a	117 ± 14.1 a
Control	15	11.5 ± 3 d	8.4 ± 2.7 d	2.1 ± .7 c	2.3 ± 1c

^aData analyzed by Proc GLM; means followed by different letters in a column are significantly different (*P* < 0.05) by Tukey's test.

^bTwenty-five replicates.

TABLE 3. RESPONSE OF RELEASED MEDITERRANEAN FRUIT FLIES TO 10 mg OF TML AND CERALURE COMPOUNDS OVER A THREE WEEK TEST PERIOD.

Treatment	N	Male fly capture (mean \pm SE)		
		Week 1	Week 2	Week 3
TML	15	111 \pm 19.4 bc	0.1 \pm .1 b	0 \pm 0 a
Com. Ceralure	15	135 \pm 16.8 b	3.1 \pm 2.2 b	0.5 \pm .2 a
(+) Ceralure B1	15	135.7 \pm 33.4 b	5.5 \pm 4.5 b	0 \pm 0 a
(\pm) Ceralure B1	15	274.5 \pm 21.8 a	79.6 \pm 33.3 a	4.7 \pm 3.7 a
(-) Ceralure B1	15	338.9 \pm 40.1 a	69.3 \pm 24.9 ab	0.3 \pm .3 a
Control	15	11.4 \pm 3.9 c	2.3 \pm 2.1 b	0.07 \pm .1

^aData analyzed by Proc GLM; means followed by different letters in a column are significantly different ($P < 0.05$) by Tukey's test.

ceralure B1, commercial trimedlure, and commercial ceralure, were not significantly greater than control. After two weeks, racemic ceralure B1 captured more males than any other compound, although the response was only 2% that of the initial response. By the third week of testing, all compounds were capturing few males flies.

DISCUSSION

In this study, we have evaluated the initial biological activity of a new stereoselectively synthesized enantiomer of the ceralure B1 isomer. The enantiopure (-)-ceralure B1 was more attractive to male medflies than either commercial ceralure (a mixture of 16 isomers), or commercial trimedlure (also a mixture of 16 isomers). This increase in relative attraction was not initially apparent from dose-response studies in the cage olfactometer (Table 1) but was clearly demonstrated in field tests at doses of 0.1–10 mg (Table 2). In addition, longevity tests with 10 mg showed greater capture in the field after two weeks compared to trimedlure or ceralure (Table 3).

From a practical perspective, these results clearly demonstrate that (-)-ceralure B1 and to a lesser extent, racemic ceralure B1, are significantly more potent medfly attractants than commercial trimedlure and ceralure. Capture by (-)-ceralure B1 outperformed the standard trimedlure used in survey and detection of medfly by as much as ninefold. Furthermore, the increased longevity of this compound, and hence the decreased volatility of this attractant compared to trimedlure, suggest that on a molecule-to-molecule basis, (-)-ceralure B1 should have even greater activity compared to trimedlure. Nevertheless, this conservative estimate demonstrates that the activity of (-)-ceralure B1 is greater than that reported for (+)- α -copaene (2–5 times more active than trimedlure) or even the

C isomer of trimedlure. To our knowledge (–)-ceralure B1 is the most potent medfly attractant thus far reported. Studies to demonstrate its effectiveness with wild fly populations have shown (–)-ceralure B1 to outperform trimedlure in the field (unpublished data). Further studies are currently in progress.

The increased effectiveness of (–)-ceralure B1 may justify the use of this attractant as a replacement of trimedlure, which is used annually in over 100,000 baited detection traps for monitoring infestations of the Mediterranean fruit fly in the United States. The effectiveness of (–)-ceralure B1 may have further implication for its potential use for male annihilation trapping of male medfly for control and eradication.

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